APPENDIX G

GUIDANCE
FOR
PREPARATION, HANDLING, AND VALIDATION
OF
PERFORMANCE EVALUATION SAMPLES

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G-1. Introduction. PE samples are an integral part of a comprehensive laboratory validation program and are used to evaluate the performance of the entire laboratory system for a specific parameter and matrix. This includes sample tracking, preparation, analysis, method selection (i.e., selection of particular options within specified standard operating procedures), record keeping, and data reduction and reporting. The USACE's HTRW Quality Assurance (QA) Program routinely employs PE samples to validate the performance of a contract laboratory and to evaluate the quality of data produced by a validated laboratory. The USACE has developed a number of PE samples in water, soil, and sediment matrices for various environmental analyses. Other new PE samples in the above matrices and air matrix are under development to fulfill the USACE’s environmental mission needs.

a. PE samples used for performance evaluation could be either single blind or double blind. A single blind PE sample is known to be an audit sample, but its composition is not known to the analyst. A double blind PE sample is intended to be indistinguishable from a routine field sample such that a laboratory will not devote more attention to produce non-routine analytical performance. Use of double blind PE samples is perhaps the most ideal approach to the assessment of laboratory performance. However, stability considerations for aqueous samples and homogeneity concerns for soil samples present substantive obstacles to the effective use of double blind PE samples. Given these concerns, use of single blind PE samples is currently the most effective and economical mechanism for monitoring laboratory performance.

b. The preparation process for PE samples should be carefully planned to ensure the precision, accuracy, and reproducibility of each batch of PE samples. Detailed preparation procedures should be documented and maintained in-house per proper USEPA and USACE guidance for legal defensibility. All chemicals, reagents, and solvents used should be pre-analyzed to ensure that they meet high purity requirements. Each gravimetric and volumetric measurement devices such as titrant, balance, and calibrant should be certified against the National Institute of Standards and Technology (NIST) standards whenever available. Only ASTM class A volumetric glassware should be used for PE sample preparation. Each batch of resulting PE samples should be checked to confirm concentrations. All PE samples should be refrigerated and stored in the dark to ensure maximum storage life.

c. Ideally, all PE samples should have the following characteristics:

- Physical similarity to field samples.

- Analyte and interference content similar to field samples.

- Analyte concentrations near the levels expected in field samples, or, in absence of this information, concentrations that span the range of the analytical method.

- Behavior similar to actual field samples throughout laboratory handling and method manipulations.

- Ability to provide useful information on laboratory performance as well as documentation of associated data quality.

d. During the design and development of PE samples, the USACE must ensure that the following goals are considered and met.

- Suitability of the materials to mimic real world environmental samples for performance evaluation of sample processing and analysis.

- Homogeneity of the materials in terms of the target analyte profile.

- Stability of the materials in terms of the target analyte profile over an extended time no less than specified holding time.

- Long-term availability of a sufficient and reliable supply of PE samples.

- Legal defensibility of the data associated with PE samples.

- Minimization of the cost and time required to produce these materials.

G-2. Determinig PE Sample Requirements and Specifications. As aforementioned, ideal PE samples should be site-specific. The constituents (analytes and matrices), concentrations, and associated acceptance limits for PE samples should be selected based on certain key aspects of the specific project: project goals and objectives, data quality objectives (DQOs), and analytical methods to be employed. However, due to the great number and broad variety types of environmental projects and

programs that the USACE is involved in, site-specific PE samples are not cost effective and may not be available in a timely manner. Furthermore, the USACE PE samples are mainly used for validation of contract laboratories prior to field sample analysis. The continuous monitoring of contract laboratory's performance during the time period of active field sample analysis is mainly achieved through analysis of split field QA samples by government QA laboratories, with supplemental PE samples if needed. Therefore, the USACE PE samples are basically designed and prepared on a non-site-specific basis.

a. Matrix. Ideally, the matrix for the PE samples should be relevant to the problem at hand and must be accurately characterized. The matrix can generally be categorized into water, soil, sediment, sludge, ash, oil, waste, etc. However, significant matrix differences can be found, for example, between two soil or even two water samples. The design of PE samples should include consideration of the origin, mineralogy, and pretreatment of the field samples. Because total site-specific PE samples are not cost effective and/or available in a timely manner, the USACE normally uses reagent water and real world soil and sediment as matrix materials for preparation of PE samples. By special requests, the USACE can also prepare PE samples with site-specific sample matrices, such as spiked field samples or spiked well-defined field matrices.

b. Methods. The analytical method or instrumentation to be used for analysis must be considered when selecting or preparing PE samples. PE samples prepared for a highly sensitive instrument, such as graphite furnace atomic absorption (GFAA) spectrophotometer, may not be appropriate for a less sensitive instrument, such as flame atomic absorption (FLAA) spectrophotometer or inductively coupled plasma (ICP) atomic emission spectrometer. Because most USACE environmental projects request USEPA SW-846 methods for sample analysis, the majority of USACE PE samples are designed and prepared for evaluation of a laboratory's capability in SW-846 methods. PE samples for the USEPA CLP or drinking water methods are also available.

c. Quality Assurance/Quality Control. The laboratory validation process is usually focused on certain specific problems with a laboratory’s quality assurance/quality control (QA/QC). With proper use of different types of PE samples, specific QA/QC problems can be detected and corrected. For example, analytical precision could be verified by duplicate PE samples that are extremely homogeneous (such as water) and contains many analytes at midrange concentrations. Matrix spike recovery problems can be verified by sending a spiked field sample and a spiked extract or digest of the same field sample.

Differences in recoveries between pre- and post-extraction/digestion spikes will demonstrate whether the laboratory's extraction/digestion process is at fault. Precision data based on PE samples of clean matrices and on PE samples of real world matrices provide information about the true laboratory precision against the precision difficulty associated with the method on complex matrices.

d. Analytes. A PE sample must contain target analytes, but it also should contain components that cause known interferences when the target analytes are measured. This approach will uncover whether or not the laboratory is performing interference correction and the extent to which the correction is effective. Sometimes the difficulties encountered by a laboratory in the analysis of PE samples may be due to limitations of a method or an instrument. When considering candidate PE samples, one should obtain as much information as possible about the analytes of interest, all possible interfering species, and the limitations of the method or instrument.

(1) It is common to include problematic and non-problematic analytes and to evaluate a laboratory's performance proficiency on an analyte-by-analayte basis. PE samples that contain problematic analytes that are unstable, reactive, or interfering under optional preparation/analysis conditions can be used to check whether a laboratory takes proper precautions and corrective actions. Examples of these include: breakdown of DDT and endrin in a dirty gas chromatography (GC) injection port; loss of dichlorobenzene (the most volatile of the semivolatile compounds) by a poor nitrogen blow-down technique; loss of phenols caused by incomplete acidification of the sample, a less than required extraction time, excess drying out of the extract, etc.

(2) False-positive problems can be identified by looking for detection of analytes that are purposely left absent. False-negative problems can be identified by adding low level analytes and watching for non-detects. Or, the PE samples may contain isomers of analytes that elute close together and share a common GC/MS ion (for example, 2,4,5 & 2,4,6-trichlorophenol, 4-nitrophenol & dibenzofuran, benzo(a)anthracene & chrysene, benzo(b) & benzo(k) fluoranthene, anthracene & phenanthrene), high level of transition metals (especially iron) that exhibit potentially interfering spectral lines, or excess phthalate esters or elemental sulfur that interferes with pesticide or PCB analysis. These conditions are designed to mimic problems that would occur in analyzing routine field samples.

(3) PE samples should be designed to evaluate the entire analytical process. Specific modifications of the composition of PE samples provide additional checks of specific procedures. For example, semivolatile PE samples should contain acid, base, and neutral extractable over the full retention time range. However, the addition of isomeric pairs to organic PE samples will check GC resolution; the addition of phthalates to pesticide PE samples will test extract cleanup methods; the addition of oil to soil PE samples will verify whether gel permeation chromatographic cleanup was performed as contract required; and the use of potassium ferricyanide, instead of potassium cyanide, to prepare aqueous cyanide PE samples will check whether distillation was conducted. Various other analytes may be added to gauge instrument performance, such as addition of chloromethane to volatile PE samples to check for correct purge flow, addition of di-n-octyl phthalate to semivolatile PE samples to determine if the GC/MS transfer line temperature was set too low, use of specific xylene isomers to indicate if proper standards and response factors were used to set up instrument criteria, etc.

(4) Certain groups of compounds should not be combined since they will react together. For example, semivolatile acids (phenols) should not be combined with bases (anilines), because these compounds will react with each other causing subsequent loss of analytes. Silver and low to medium levels of chloride are incompatible and should not be mixed. Certain compounds may not even be compatible with some instruments and should not be used. For example, it is difficult to use GFAA to analyze a PE sample with a high concentration of chloride because of analyte signal suppression.

G-3. Preparation of PE Samples. PE samples can be prepared either by spiking known amounts of analytes into a well defined homogeneous matrix or by defining well homogenized real world samples. The USACE PE samples can generally be categorized into two groups based on preparation methods: fortified PE samples and "real world" PE samples. The fortified PE samples are prepared by spiking high purity reagent water or control solid materials with solvated target analytes of high purity. Fortified PE samples cost less to prepare and allow qualitative and quantitative variations in the compositions of final PE samples. Real world PE samples are usually soil or sediment collected from contaminated sites, which are dried, ground, mixed, and analyzed prior to use. Real world PE samples are used for validation of laboratory performance in soil analysis for two reasons: (a) it is very difficult to prepare an absolutely homogeneous sample that can then be subsampled for PE samples and (b) a spiked sample can never truly represent the weathering and complexities

of a naturally contaminated matrix. Because the constituents are integrated into the matrices as naturally as possible, the real world PE samples present special analytical challenges of matrix interferences. The USACE is continually seeking suitable real world samples that represent typical environmental samples and contain a broad spectrum of target analytes at adequate concentrations.

a. General Preparation Procedure. Regardless of the type of PE samples, the general USACE procedure for preparing PE samples is outlined below.

(1) Determine matrix type, analytical method, and instrumentation.

(2) Calculate the amount of PE samples needed by volume or weight.

(3) Select analytes, interferences, solvents, and preservatives.

(4) Decide on the concentration of each component.

(5) Select stock materials and calculate appropriate amounts to add.

(6) Write step-by-step instructions (i.e., standard operating procedures).

(7) Perform an error analysis and determine performance requirements.

(8) Obtain materials.

(9) Prepare the PE sample.

(l0) Verify the concentration of each component in the PE samples.

(11) Verify PE samples by multi-laboratory referee analyses.

(12) Establish the performance acceptance limits of PE samples.

When real world materials are used for preparation of fortified or non-fortified PE samples, additional intra- and interlaboratory analyses are needed to verify the compositions of the real world materials. Any indigenous levels of analytes and interferents that are present in the real world materials must be

accurately determined. Depending on the levels and types of analytes and interferents, the real world materials may be used for preparation of fortified or non-fortified PE samples.

b. Starting Materials and Stock Solutions. Starting materials and stock solutions must fulfill several criteria in order to be suitable for preparation of PE samples. All critical information about starting materials and stock solutions should be recorded in logbooks such that the PE samples are traceable to NIST or other reliable reference materials.

(1) Purity is the first requirement, especially if the final sample's true values are going to be based on the material added to the sample. Only chemical sources of known high quality will be used for PE sample preparation. The purity of all reagents, acids, and solvents should be checked prior to use. Purity is not as much of a factor if the PE sample is going to be characterized with consensus values from reputable laboratories.

(a) For inorganic PE samples, contaminant levels should be in the low ppm range if the PE sample is to contain only one analyte. Higher purity (low ppb range) starting materials should be used if multianalyte PE samples are prepared (to avoid contamination) or if sensitive instrumentation is used. The amount of target analytes in the starting material should be certified to within ±0.5 percent for most cases. Materials that are sold without certified purity information should not be used. Individual metal solutions are either purchased from NIST or from vendors whose materials are traceable to NIST.

(b) For organic PE samples, only the highest purity solvent should be used. Purge-and-trap grade methanol is necessary for preparation of volatile spiking solution because lower grades frequently contain toluene and xylene as impurities. Standards for use in preparing organic PE samples may be purchased neat, as single component solutions, or as multiple standard mixes from reliable vendors.

(2) Stability and chemical compatibility are other important criteria for starting materials and stock solutions. Specific reagents for each analyte are selected on the basis of availability and chemical characteristics, such as stability and reactivity. An expiration date must be specified for all prepared materials.

(3) For solid PE samples, unless the entire sample is to be analyzed, homogeneity is one of the most important factors to be considered. Natural solid matrices, such as soil or sediment, should always be dried, ground, sieved, and mixed thoroughly

prior to spiking. For solid PE samples, the smallest sample aliquot that will provide reproducible analysis results could be estimated with Pierre Gy's sampling theory and confirmed by replicate analyses. (See F. F. Pitard, Pierre Gy's Sampling Theory and Sampling Practice, 2 volumes, 1989, CRC press, Inc., Boca Raton, Florida.) For liquid PE samples, homogeneity is inherent unless adhesion of analytes to the container wall or multiple phases are present. Normally, multiple phase PE samples should be avoided because sampling errors may overwhelm all other errors, thus limiting a study's usefulness.

(4) Starting materials and stock solutions should also be obtained at appropriate concentration levels to minimize the amounts required and remain in the realm of accurate laboratory ware measurements. For example, weights of solid materials should be between 0.1 and 500g and volumes of liquids should be between 50 µL and 500 mL. Avoid dilutions that would require odd sizes of volumetric ware. If several levels for a given analyte are available, the more concentrated solution should be chosen to minimize potential contamination from stock materials.

(5) When commercially available reference materials are utilized, only certified reference materials (CRMS) should be used for PE sample preparation. The term "certified" means that documentation supports the reference material. Using a CRM may assure the capability of the measurement system to determine the analyte in the sample. NIST is the most widely used supplier of CRMs. However, using NIST values for solid materials can lead to comparison errors on data obtained using USEPA inorganic and organic extraction methods. NIST expresses CRM values as "total" concentrations but many USEPA methods use values based upon "extractable" concentrations. Because of this, certified NIST values for solid CRMs usually cannot be used. Using NIST values do not pose a problem in performance evaluation of laboratories for water analysis, where "total" extractables approximate true values. The acceptance criteria of the USACE PE samples should be established based on extractable concentrations.

(6) Each lot of standards used for preparation or verification of PE samples should be analyzed by the PE sample suppliers to verify its concentration prior to use. Reanalysis of the standards is required periodically to verify stability, according to a schedule optimized for each standard. The probable error of each step in the preparation of PE samples should be evaluated and used to assess the overall probable error and implications on confidence levels. Details analysis and validation procedures should be documented and maintained in-house per appropriate USEPA and USACE guidance for legal defensibility.

(7) All PE sample suppliers must actively participate in State and/or Federal proficiency testing programs and provide the USACE Laboratory Validation Committee with their most recent results for review on a quarterly basis.

(8) Safety must also be considered. Material Safety Data Sheets (MSDS) should be obtained with each material and should be read and followed carefully. As good laboratory practice, handle and weigh out all toxic materials in a well ventilated fume hood.

c. Calculations. The calculations involved in preparing fortified PE samples are relatively simple. It is best to start with the final volume or weight of PE samples to be prepared and work backward to determine the amounts of individual analyte stocks needed. Care should be taken in calculations with reagent purity values, gravimetric factors, dilution and concentration factors, significant figures, and unit manipulations. The most common types of concentration units are weight/weight for solid PE samples and weight/volume for liquid PE samples. Reagent bottles should be labeled with specific units, such as µg/mL or µg/kg. Ambiguous units such as ppb or ppm should not be used because these units do not differentiate between weight/weight or weight/volume. Depending on when they are noticed, calculation errors can have serious ramifications when PE samples are involved. Therefore, it is best to double check all calculations leading up to the final concentrations of PE samples before the samples are prepared. Good laboratory practice should include a second person's review of the calculation.

d. Standard Operating Procedures. In order to prepare reliable PE samples, adherence to prescribed preparation procedures is imperative. In any operation that is performed on a repetitive basis, reproducibility is best accomplished through the use of standard operating procedures (SOPs). This is especially true for preparing PE samples that will be used to determine laboratory performance. An SOP is defined as a written, narrative, and stepwise description of laboratory operating procedures including examples of laboratory documentation. An SOP should accurately describe the actual procedures used in the laboratory to ensure that reproducible results can be achieved by following the SOP. The SOP for PE sample preparation should be prepared as part of the planning process and should be at or near completion before PE sample preparation work begins. The SOP should be reviewed before preparing actual PE samples. Ambiguous statements or terminology like "air dried at ambient temperature" or "1:10 dilution" should not be used when "18 to 22°C" or "ten-fold dilution" is meant. The "l:l0" may be confused with one part concentrate diluted with ten parts of diluent, which is really an 11-fold dilution. As a

PE sample is prepared, changes and observations are documented so that significant information will be available if needed later.

e. Fortified PE samples. Fortified PE samples are usually prepared with spiking techniques. Either large volumes or small units of fortified PE samples of any matrix can be prepared by spiking analytes of choice at selected concentrations. Normally, it is preferable to spike a large volume and create individual units from it, unless there is a major concern of analyte loss to container wall. PE samples should be prepared by designated, experienced senior chemists to improve batch-to-batch reproducibility and reliability.

(1) Fortified aqueous PE samples. Aqueous PE samples should be prepared on the day of shipment, usually early in the week to allow adequate preparation time for the contract laboratory to perform digestions, extractions, cleanups, etc. before the weekend.

(a) Reagent water which is free of contaminants at the method detection limits is normally used for PE samples preparation. Reagent water can be prepared by passing tap water through a reverse osmosis water system and then through an ultraviolet and activated carbon cartridge or equivalent system to produce analyte-free reagent water. The quality of reagent water should be monitored and documented on a routine basis.

(b) ASTM class A pipets and calibrated microsyringes should be used for delivering and spiking during PE sample preparation. Variable pipetters can be used if they are verified to be in calibration; however, glass pipets are preferred. Sample containers (high density polyethylene for inorganic and amber glass for organics) are purchased as “certified pre–cleaned” according to USEPA standards.

(c) Gravimetric measurements can be used on less volatile liquids, such as water. If weights are used for calculations, density of the liquid also must be determined so that weight-to-volume units can be calculated. Volatile liquids have to be prepared by volume, using minimal headspace and minimal exposure to the atmosphere. Diluents should already contain any required preservatives so that final volumes are not altered by preservation.

(d) Full-volume PE samples of one liter are normally used for aqueous organic PE samples except volatiles which are 40 mL. A trip blank should always accompany volatile samples for each different analytical method. Volumes for the inorganic analyses

vary from 200 to 1,000 mL, depending on the target analytes and analytical methods.

(e) Multiple sets of spiking solutions are maintained with varying constituents and concentrations to avoid sending the same PE samples to the same laboratory twice or to affiliated laboratories of the same parent organization.

(f) Organic spiking solutions (except volatiles) are prepared by dilution of reference stocks. Records and certificates of all stock solutions and dilutions are maintained in standard logbooks. Aqueous PE samples for organics (except volatiles) are spiked individually into the sample bottles since the entire sample is used for analysis.

(g) Volatile spikes are purchased as mixed solutions designed for laboratory evaluations and certificates are maintained in laboratory files. Aqueous volatile PE samples are prepared in a volumetric flask with sufficient volume to prepare the day's shipment and then transferred to 40-mL VOA vials for submission to contract laboratories.

(h) Aqueous PE samples for inorganic are prepared in volumetric flasks and aliquots are then transferred to individual sample bottles for shipment.

(i) All PE samples should be properly preserved per method requirements. PE samples with critical holding times should be shipped immediately after preparation to allow adequate time for the contract laboratory to prepare and analyze the PE samples.

(j) Only one aliquot of each aqueous PE sample will be sent to each contract laboratory. Because the aqueous PE sample is prepared with reagent water, the laboratory will be instructed to perform method-specific QC analyses with its own reagent water.

(2) Fortified solid PE samples. Various types of soil samples are collected and prepared to serve as a solid matrix. The soil could be clayey, silty, or sandy with different alkalinity, organic, and metal contents. However, care must be taken to avoid using soils that are very reactive to acids or other reagents used for sample preparation. Except for volatile organics, solid PE samples can be prepared by solid or liquid addition. Due to the high volatility of volatile organics, soil PE samples for volatile organics can be prepared by a vapor fortification technique. (See A. D. Hewitt, P. H. Miyares, D. C. Leggett, and T. F. Jenkins, *Comparison of Analytical Methods for Determination of Volatile Organic Compounds in Soils,* Environ.

Sci. Technol., **1992,** 26, 1932.) The USACE is looking into this technique.

(a) After removal of extraneous materials such as rocks, sticks, etc., the soil will be air dried, ground, and mixed with mills or grinders. Mixing mills or grinders capable of grinding and mixing large volumes of soil (up to 1 gallon) per batch are preferred. Separate batches can be combined, sieved to pass 150 mesh (<100 µm), and blended in a larger container. A 1 g sample aliquot should have a relative sampling error of about two percent at this particle size if the total batch is 100 g. The grinding and mixing times are established by short interval runs and examining the particle size and physical consistency of the soil. The homogenized soils should be stored in a cool, dark, and dry place. If needed, the potential influence of laboratory relative humidity can be removed by conditioning an air dried, sieved, and thoroughly mixed soil with $CaSO_4$ desiccation.

(b) The concentrations of any target analytes and interferents in the homogenized soil should be thoroughly and accurately determined. It is preferred that the concentrations of natural contaminants in the soil are below method detection limits or relatively low compared with the concentrations of spiked analytes.

(c) The spiking can be done by solid addition. The two solids that are to be mixed should be reduced to approximately the same small particle size (at least <150 mesh) before mixing. This reduction leads to easier blending and components will be less prone to segregate during storage and transit. Relative amounts of each component should not be extreme because it is very difficult to evenly distribute small amounts of one material within large amounts of another. If extremes in relative amounts cannot be avoided, the blending can be done in stages. That is a small quantity of the main component can be spiked and blended, then mixed and blended with the rest of the main component.

(d) The spiking can also be done by liquid addition. Analyte solutions (except volatiles) are sprayed over the homogenized soil in small increments. After vaporization of the solvent, mix the soil thoroughly and spray again. The above process is repeated until all analyte solutions are used up. Rinse the spray bottles with more solvent and spray over the soil again to ensure all target analytes are quantitatively transferred to the homogenized soil. When liquid spikes are used to modify solid matrix, the solvent must be removed by drying. Since local deposits of analyte can be left after drying, thorough mixing after drying is crucial. Mixing can be improved by using enough solvent to form a runny paste or mud. The paste

is occasionally stirred while drying and, when completely dry, must be re-ground and blended.

(e) Prior to packing the homogenized bulk PE samples into small units for use, the homogeneity of the PE samples should be reassessed to determine the minimum subsample size for each target analyte. A general approach is first selecting aliquots from the homogenized bulk PE samples and measuring the concentrations of target analytes. A two-way analysis of variance is then carried out by comparing results from aliquots within subsamples with those between subsamples. If the means do not differ significantly at 95 percent confident level, the bulk PE samples is considered homogeneous. Homogeneity could further be assessed by analyzing aliquots from certain percentage of the individual subsamples at a variability of, say, five percent relative standard deviation. Not all target analytes need to be tested, and a single measurement technique may be used, However, the selected analytes and technique should include be representative and conclusive.

f. Real world PE samples. Real world soil and sediment PE samples are collected from locations that have significant levels of numerous contaminants of concern. Numerous low levels of analytes that may cause problems in assessing laboratory performance should be avoided. Large volumes of materials are collected and shipped to USACE PE sample suppliers for processing. The materials are mixed thoroughly and extraneous materials are removed. Approximately five to ten gallons of materials are air dried to three to four percent moisture. The materials are then ground in a large volume grinder to pass through a 0.5-mm sieve. Materials are mixed and passed through the grinder a second time to desired particle size (i.e., 45-75 µm) and stored at 4°C in the dark.

(1) Extraneous materials such as rocks, sticks, etc. should first be removed from the solid materials. The materials are then air dried, ground, and mixed with mills or grinders. Mixing mills or grinders capable of grinding and mixing large volumes of soil (up to one gallon) per batch are preferred. Separate batches can be combined, sieved to pass 150 mesh (<100 µm), and blended in a larger container. A l-g sample aliquot should have a relative sampling error of about two percent at this particle size if the total batch is 100 g. The grinding and mixing times are established by short interval runs and examining the particle size and physical consistency of the soil. The homogenized soils should be stored in a cool, dark, and dry place.

(2) The content of natural PE samples can be altered by spiking to fulfill special needs. The same spiking technique as

previously described can be used. After spiking and drying, an additional blending step is necessary.

(3) Most real world PE samples used by the USACE are very stable. The stability of PE samples should be studied and monitored by analyzing random PE samples of each production batch according to a proper kinetics-based schedule. Some real world solid PE samples have been used as long as five years with no significant changes in concentrations in metals and semivolatile organics.

(4) Multiple sets of real world PE samples with different constituents and/or concentrations should be available and ready for use to avoid sending the same PE samples to the same laboratory twice or to affiliated laboratories belonging to the same parent organization.

G-4 . Handling. All PE samples should be handled and stored with extreme care to ensure the sample stability, integrity, purity, and authenticity.

a. Generally, containers are selected for their inertness to their contents and their ability to prevent sample loss. Samples for organic analyses are stored in amber glass to avoid the plasticizers and organics found in plastic containers. Amber glass is recommended since some analytes are ultraviolet (UV) light sensitive. Plastic bottles are suggested for metals to avoid leaching of trace impurities from glass containers. Bottle caps should be tightly closed to avoid leakage during shipment.

b. A PE sample must maintain its stability. If values change significantly before the sample can be analyzed, the PE sample is worthless. Short holding times are common practice for unstable species such as mercury, cyanide, and volatile organics. In addition to observance of holding times, preservatives and refrigeration are used to retard sample degradation. In addition, PE samples for cyanides and organic analysis should be kept in the dark to avoid degradation by UV light. PE samples must be preserved according to the required analysis. For example, aqueous PE samples for volatile organics should only be acid preserved depending on the analytical method to be used. Normally, all PE samples should be preserved and stored at 4°C in the dark to retard degradation processes. Analytes requiring different preservatives cannot be grouped together in the same sample container. Bottles for volatile organic samples should be completely filled to retard loss of volatiles.

c. All PE samples should be appropriately preserved, packed, and shipped by overnight express delivery service to commercial

laboratories according to USEPA, USACE, and DOT regulations and guidelines. Chain-of-custody form should be used for all PE samples.

d. PE samples are usually provided as single blind, although double blind are occasionally provided. When double blind PE samples are shipped, special precautions on labeling and packing should be taken to make the PE samples indistinguishable from regular field samples. The packaging and container must be identical with that used by field personnel sending the same sample type to the contract laboratory. Special arrangements, such as arranging for a "consulting firm" to contract with the laboratory to be evaluated or using the same bottles, labels, chain-of-custody forms, sample coolers, shipping location, etc. as used in the field, will be made to simulate actual environmental samples.

G-5 . Validation. Because PE samples may be used to disqualify a laboratory's performance or to challenge a laboratory's results, the analyte concentrations in PE samples must be validated with legal defensibility prior to use. All PE samples should be meticulously tested internally and externally to determine the true values and statistically establish the acceptance limits prior to use.

a. Two approaches, the consensus interlaboratory approach and the multiple techniques/definitive techniques approach, are usually used for validation of PE samples. In the multiple techniques/definitive techniques approach, the PE samples are tested by independent techniques with different measurement principles and by definitive techniques whose measurement principles are based on or are directly traceable to physical measurements such as weight and radioactive decay to reduce random or systematic variabilities of chemical measurement techniques. Nearly all of NIST's environmental standard reference materials are certified by this approach. However, there are few definitive techniques and none for organics. The majority of USACE PE samples are validated by interlaboratory consensus in performing a single methodology where the mean value approximates the true value. When there is no definitive technique available to check, the mean value obtained by interlaboratory consensus could be nothing more than a statistical average. Therefore, only reliable laboratories of high performance should be used for validation of PE samples.

b. Fortified PE samples. Depending on the type of fortified PE samples, the true concentrations and acceptance limits of each target analyte can be determined by three different methods:

referee laboratory analysis, error propagation analysis, or performance data estimation.

(1) Fortified aqueous PE samples. The true values and acceptance limits of fortified aqueous samples can be determined by all three methods. Normally, consensus values by referee laboratory analysis should be used. If the other two methods are used, a triplicate for each batch of PE samples should be analyzed by the USACE PE sample supplier to check the accuracy and precision.

(a) Referee laboratory analysis. For analytes with critical holding times, PE samples should be sent to the contract laboratory being tested at the same time they are sent to a minimum of four referee laboratories. The uncertainty of the mean value based on referee laboratory's results decreases with increasing number of laboratories. Therefore, it is preferred to have more laboratories (e.g., 12 referee laboratories) to improve the confidence level of the mean value. The determined concentration from each independent referee laboratory should be within ten percent of prepared concentrations or the causes of excess high/low recovery should be investigated. Consensus values within 95 percent confidence level from the referee laboratories can then be used for evaluation of the contract laboratories. Stable analytes can be characterized before shipment to contract laboratory.

(b) Error propagation analysis. If a material is not characterized (i.e., round-robin data not available), acceptance limits can be calculated. Sometimes calculation is the only way to determine the true values and acceptance limits. The calculation for the expected or true concentration for each analyte in fortified aqueous PE samples is very accurate and straightforward. The acceptance limits of fortified aqueous PE samples can be determined through an error analysis of the steps caused by analytical sample preparation and by sample analysis. Error propagation rules are used as guidelines to estimate determinate and indeterminate errors that should be experienced by the laboratory being evaluated. The indeterminate errors are always judgement calls and should be based on experience. The Factor-2 criterion (i.e., indeterminate errors = 2 x determinate errors) can be used as a good approximation for inclusion of indeterminate errors. The result is a relative error that can be multiplied by the expected target values for each analyte to get acceptance limits. If biases are known to exist but cannot be reliably accounted for, the PE sample may have to be characterized by several reputable laboratories and consensus values used for acceptance windows.

(c) Performance data estimation. The performance data for a number of USEPA methods, based on multiple laboratories testing results, are published in the methods. The acceptance limits for each analyte can therefore be estimated by the calculated target values and the precision formula. The estimated acceptance limits usually are very reliable.

(2) Fortified solid PE samples. A difficulty with fortified solid PE samples is matrix interaction with the analytes. Analyte accuracy of the spiking solution may be very well known, but that accuracy is lost after spiking, when the analytes react with the solid matrix. For example, adsorption of metal ions in solution by the clay matrix of a soil is a well known phenomenon. Since most USEPA extractions are designed to remove leachable rather than true totals, all the analyte that was introduced by spiking may or may not be removable by the sample preparation method. The result is a reduced recovery for affected analytes. Analytes like antimony, silver, and selenium are especially susceptible. To complicate matters further, if indigenous levels of analytes are present in the solid matrix, their leachable levels must be known before total levels or percent recovery can be calculated accurately. Given these difficulties, a fortified solid PE sample is best characterized by consensus rather than by calculation or estimation of analyte levels from individual components.

c. Real world PE samples. For real world PE samples, the true values of target analytes are usually unknown. The mean of reported values from a round-robin testing is usually considered a "consensus" value and would be used as the true analytical value. Confidence intervals for the consensus values of target analytes are based on reported values using standard population statistics. The initial acceptance limits for PE samples are statistically determined by consensus values of the participating laboratories which include reputable government and contract laboratories. The acceptance limits for each target analyte will be established statistically at 95 percent confidence level. The acceptance limits for each target analyte are matrix- and method-specific.

(1) Any method of evaluating real world PE samples may present problems of accuracy that depend upon the amount of data used to set acceptance limits. Thus, it would be best to send split PE samples to a minimum of four round-robin testing laboratories. Although a consensus value resulting from a small number of determinations may have significant uncertainties, the consensus value from the round-robin testing laboratories should be a better estimate of true value than any single measurement.

(2) A round-robin analysis is used to certify analytes of interest. In order to ensure the integrity of PE samples, one or two PE samples should periodically be resubmitted to the referee laboratories to evaluate any possible degradation or trends in the analyte concentrations. This information is also used to evaluate possible extension of the useful life of real world PE samples.

d. The pool of PE sample results produced by all contract laboratories should be carefully analyzed on a regular basis. The mean values and the associated uncertainties of target analytes should always be documented. The program-wide statistical results for PE sample analyses by contract laboratories should also be used to adjust the acceptance limits in order to observe the relative performance of each laboratory using a given protocol against its peers. The USACE may adjust the acceptance limits on any given PE sample to compensate for unanticipated difficulties with a particular sample or analysis.

e. All PE samples must be analyzed with the same methodology (i.e., USEPA SW-846 of the most recently promulgated revisions) by both the contract laboratories and the referee laboratories. Deviations from the standard methods will make the data noncomparable. The results of all PE sample analyses should be used to develop control charts displaying the true concentration and ranges of recovery and bias for each target analyte.